Establishing temperate crustose Early Holocene coralline algae as archives for palaeoenvironmental reconstructions of the shallow water habitats of the Mediterranean Sea

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Abstract

Over the past decades, coralline algae have increasingly been used as archives of palaeoclimate information due to their seasonal growth bands and their vast distribution from high latitudes to the tropics. Traditionally, these reconstructions have been performed mainly on high latitude species, limiting the geographic area of its potential use. Here we assess the use of temperate crustose fossil coralline algae from shallow water habitats for palaeoenvironmental reconstruction to generate records of past climate change. We determine the potential of three different species of coralline algae, *Lithothamnion minervae, Lithophyllum stictaeforme* and *Mesophyllum philippii,* with different growth patterns, as archives for pH ($\delta^{11}B$) and temperature (Mg/Ca) reconstruction in the Mediterranean Sea. Mg concentration is driven by temperature but modulated by growth rate, which is controlled by species-specific and intraspecific growth patterns. *L. minervae* is a good temperature recorder, showing a moderate

warming trend in specimens from 11.37 cal ka BP (from 14.2 ± 0.4 °C to 14.9 °C ± 0.15) to today. In contrast to Mg, all genera showed consistent values of boron isotopes ($\delta^{11}B$) suggesting a common control on boron incorporation. The recorded $\delta^{11}B$ in modern and fossil coralline specimens is in agreement with literature data about early Holocene pH, opening new perspectives of coralline-based, high-resolution pH reconstructions in deep time.

Key words: coralline algae, Holocene, Mediterranean Sea, *Lithothamnion*, *Lithophyllum*, *Mesophyllum*

Introduction

Shelf sea benthic ecosystems are among the most productive and diverse on the planet, but are increasingly impacted by global change (Hall 2002). Direct consequences of the increase in anthropogenic atmospheric CO_2 are warming and acidification of the ocean (Pörtner *et al.* 2014). Coastal areas are highly variable environments whose dynamics are heavily driven by local factors, *e.g.* riverine discharge, local bathymetry and coastline morphology. Our ability to predict current and future human influences on climate and ecosystem requires knowledge of climatic histories, especially from the most recent past (Hönisch *et al.* 2012).

Even though it is not possible to measure the key physical and chemical variables of the past environments directly, their reconstruction is still possible by "proxy" (Henderson 2002). Climate proxies use preserved chemical characteristic in the sediment and organisms of the past that have responded systematically to changes in environmental variables such as pH and temperature.

The majority of the deep time studies of environmental reconstructions have focussed on the open ocean (e.g. Lear *et al.* 2000; Hönisch *et al.* 2012b; Seki *et al.* 2010; Yu *et al.* 2014) with fewer studies analysing the environmental changes in shallow waters. In these enviroments, the potential of organims with incremental growth patterns has been used to generate high resolution records of seasonal climate including bivalves (Wanamaker Jr *et al.* 2011; Wanamaker *et al.* 2009), coralline algae (Halfar *et al.* 2000; Fietzke *et al.* 2015; Cusack *et al.* 2015; Burdett *et al.* 2011; Chan *et al.* 2011) and corals (Brachert *et al.* 2006; Pelejero *et al.* 2005).

Coralline algae (Rhodophyta, Corallinophycidae) are a common feature of modern and past continental shelves (Riosmena-Rodríguez *et al.* 2017) and contribute significantly to carbonate platform deposits from the coast to the outer shelf (Aguirre *et al.* 2017). Today, coralline algae are the most common (Foster 2001; Foster *et al.* 2013) and widespread framework builders north and south of the low latitude coral belt (Freiwald and Henrich 1994). They increase benthic diversity by providing a hard substrate with a complex architecture (Guillou *et al.* 2002; Wilson *et al.* 2004) and are a significant component of the global carbon system (Mackenzie *et al.* 2004) accounting for approximately 25% of $CaCO_3$ accumulation within coastal regions (Martin *et al.* 2007).

Coralline algae have an excellent fossil record and consistent phylogenetic reconstructions with a phylogenetically-supported morphology based on order of appearance and robust molecular clocks (Aguirre *et al.* 2010; Rösler *et al.* 2017). Their fossil record starts in the Valanginian (Chatalov *et al.* 2015) providing a unique opportunity to reconstruct shelf ecosystems. They progressively diversified, becoming one of the most important groups of framework builders and carbonate producers of the Cenozoic (Braga *et al.* 2009; Coletti *et al.* 2018). Especially since their diversification in the Miocene, abundance data is available (Halfar and Mutti 2005). As such, they have the potential to reveal changes in seasonal climate change in coastal sections of the Miocene (Braga *et al.* 2009) and the Paleocene-Eocene Thermal Maximum (Scheibner and Speijer 2008). Therefore, over the past decades, coralline algae have increasingly been used as archives of palaeoclimate information, as they are excellent carrier organisms for reconstructing centennial-length palaeotemperature records due to their high-Mg calcite skeleton (Chave and Wheeler 1965; Halfar *et al.* 2000; Hetzinger *et al.* 2009; Kamenos *et al.* 2008; Kamenos 2010; Caragnano *et al.* 2017; Fietzke *et al.* 2015). However, the potential of corallines as palaeoclimate recorders has not been tested yet for times older than the XIV century (Kamenos 2010).

Despite their broad distribution, only a few high latitude, cold-water species such as *Lithothamnion glaciale* (Kamenos *et al.* 2008), *Clathromorphum nereostratum* (Hetzinger *et al.* 2009) and *Phymatolithon calcareum* (Kamenos *et al.* 2008) have been used for palaeoreconstruction to date. In contrast, sub-tropical and tropical species such as *Mesophyllum crassiusculum* (as *Lithothamnion crassiusculum* in Halfar *et al.* 2000) and *Lithophyllum yemenense* (Caragnano *et al.* 2014, 2017) have been less used, and not a single temperate species is established as a palaeo-proxy archive. In some parts of the Mediterranean Sea though, coralline fragments are the dominant component of sediments, comprising over 80%

of the seafloor between 10 and 70 meters depth and 15% between 70 and 100 meters (Bracchi and Basso 2012) and hence they hold significant potential as palaeoevironmental recorders.

Most of the above-mentioned commonly used species form rhodoliths (*L. glaciale, L. crassiusculum, L. yemenense* and *P. calcareum*) or thick crusts with strong vertical accretion (*L. yemenense* and *C. nereostratum*), whereas the most common species in the temperate seas form crusts that expand horizontally with moderate thickening of the thallus. Such differences in growth pattern may influence their behaviour as proxy archives and need to be rigorously assessed (Adey *et al.* 2013; Halfar *et al.* 2011; Caragnano *et al.* 2014).

Here we investigate the potential of three widely distributed taxa of temperate coralline species with different growth patterns, to record temperature and pH in recent geological history. We are using the Mediterranean as a testing ground due to the well understood regional differences in Holocene climate *e.g*. the timing of the Holocene climate optimum (Cacho *et al.* 2001). We are focusing on one of the Mediterranean cold spells TC5-TC6 (9.87-11.56 ca ka BP, Cacho *et al.* 2001) and contrast this with "background" conditions. We consider three widely distributed taxa collected in the western Mediterranean Sea: *Lithothamnion minervae* Basso (growth pattern: mainly thickening-T), *Lithophyllum stictaeforme* (Areschoug) Hauck (growth pattern: mainly marginal elongation-ME), and *Mesophyllum philippii* (Foslie) Cabioch & Mendoza (growth patterns: both marginal elongation and thickening-ME&T) (Fig. 1).

Material and Methods

Geological settings

The Mediterranean Sea has a complex geological history. Because it is a near-landlocked body of water in a dry climate, the basin undergoes intensive evaporation (Mariotti 2010; Romanou *et al.* 2010). Its water budget is presently negative, and constantly compensated by Atlantic water influx (Romanou *et al.* 2010) though the salinities are generally slightly above the Atlantic Ocean (36.18‰ in the Atlantic vs 36.8-38.7‰ in the Mediterranean, increasing from the Alboran Sea to the Levantine Basin; Lacombe and Tchernia 1972). The Mediterranean has a well-documented sedimentary record of astronomically-induced climatic variations (Krijgsman 2002; Emeis and Sakamoto 1998). The basin underwent several sea-level fluctuations that shaped the Mediterranean coastline and the shelf profile. During the Last Glacial Maximum (LGM) sea-level was about 130 m below the present one, similar to the global sea level change (Lambeck *et al.* 2004; Lambeck_and_Purcell 2005). In response to

global ice melt, sea level rose in several steps. During the early Holocene, 11,650 yrs BP and 7000 yrs BP sea level rose rapidly. After ca 7000 BP the overall sea-level rise decreased of an order of magnitude (Lambeck *et al.* 2004). The flooding of continental shelves since the LGM was the most important geological event of recent time.

The Pontine Archipelago consists of five islands, subdivided into western (Palmarola, Ponza and Zannone Islands) and eastern (Ventotene and Santo Stefano) groups. Palmarola and Ponza are divided by a NS trending channel 7 km wide and with a maximum depth of 70 m. The archipelago is a Plio-Pleistocene volcanic structure located on the external margin of the continental shelf in the central Tyrrhenian Sea (Fig. 1), with a shelf-break situated at a depth of 105–160 m suggesting partial exposure during the LGM. In the south-western sector, the continental slope, surrounding the western Pontine Archipelago, connects the continental shelf to the Vavilov abyssal plain (Chiocci *et al.* 2003). In this area the continental slope is extremely steep and characterized by large channels (Chiocci *et al.* 2003). The Pontine volcanoes are the northern termination of a volcanic alignment that extends from the seamounts of the Southern Tyrrhenian Sea to offshore Central Italy. These represent remnant arcs that were constructed across the Tyrrhenian Sea during the eastward migration of the Ionian subduction system (Peccerillo 2017). The volcanic activity of the archipelago started in the early Pliocene and lasted until 33 Ky ago (Bellucci *et al.* 1999). The western group is mainly composed of rheolytic to trachytic subaerial and submarine volcanic units separating the two intra-slope sedimentary basins of Ventotene and Palmarola (Barberi *et al.* 1967; Zitellini *et al.* 1984; De Rita *et al.* 1986). The eastern group represents the subaerial portion of a submerged stratovolcano, emplaced at the centre of the Ventotene basin, characterized by pyroclastic and effusive products having a basaltic-trachytic composition.

Sample collection and age determination

During the summer and autumn 1989, two cruises of the R/V Minerva sampled the sea floor around the Palmarola and Ponza islands and more than 300 grab, dredge and core samples were collected (Fig. 2). Living coralline algae specimens were selected from grab and dredge samples collected at 48 m (PIC92), 44 m (PIC113), and 97 m depth (DG13) (Table 1; Fig. 2). Additionally, fossil specimens of *L. minervae*, *L. stictaeforme* and *M. philippii* were selected from the gravity cores GRC06, levels C and D and GRCO4 level D (Table 1; Fig. 2) (Basso *et al.* , 2006). AMS radiocarbon dating of coralline fragments was undertaken at the ANSTO Physics Division in Menai, Australia. Calibrated radiocarbon ages were calculated following

Stuiver & Reimer (1993) (Basso *et al.* 2006). The ages of the core layers result as follows: *M. philippii*, 8.6 ± 50 cal ka BP; *L. stictaeforme* 8.6 ± 50 and 11.37 ± 50 cal ka BP; *L. minervae* 10.6 ± 50 and 11.37 ± 50 cal ka BP (Basso *et al.* 2006). Scanning Electron Microscopy (SEM; CamScan-CS-44) was used to explore possible diagenetic alteration of the algal Mg-Calcite thallus prior to any preparation. SEM images did not show any structural evidence of diagenetic alteration such as micritized segments (microcrystalline carbonates less than 4 μm in size) of the thallus in the analysed samples (Macintyre and Reid 1995).

Mg/Ca determination-temperature reconstruction

Mg/Ca is extensively used as a temperature proxy in coralline algae and other calcifying organisms (e.g. Barker *et al.* 2005; Fietzke *et al.* 2015; Kamenos *et al.* 2008; Halfar *et al.* 2008).

Element to calcium rations in carbonate minerals depend on (eq1):

$$D_{El} = (Mg/Ca)_{mineral} / (Mg/Ca)_{seawater} \quad (1)$$

Where D_{El} is the empirical homogeneous distribution coefficient calculated on the molar concentration ratios of Mg/Ca calcite and seawater. At equilibrium, the partitioning constant between the pure mineral phases depends on temperature since the substitution of Mg into calcite is associated with a change in enthalpy. As the substitution of Mg into calcite is an endothermic reaction, the Mg/Ca ratio is expected to increase with increasing temperature (Rosenthal *et al.* 1997; Mucci and Morse 1990). Rosenthal *et al.* (1997) and Lea *et al.* (1999) suggested an exponential temperature dependence of Mg uptake into calcite of ~ 3% per °C.
Longitudinal sections of the algae were embedded in resin (Epoxy, Buehler), polished and carbon–coated for element analysis. Elemental distribution was mapped with a JEOL JXA 8200 "Superprobe" using an accelerator voltage of 15.0 kV, a beam current of 50 nA, beam diameter 2 μm, dwell time 20 ms, and 10 accumulations (GEOMAR, Kiel). Parallel transects of Mg and Ca were taken along the direction of maximum thickening (growth) and then averaged in a single record. The transects did not include the hypothallus of the specimens (Nash and Adey 2017). Elemental concentrations were calibrated against standards for Mg (glass VG-2 Juan de Fuga = NMNH 111240, Percentage of analytical uncertainty from calibration: 0.4%) and Ca (Calcite = NMNH 13632, Percentage of analytical uncertainty from calibration: 0.5%).

To avoid uncertainties from sub-annual dating, the maximum and the minimum value of molar Mg/Ca representing summer (high temperature) and winter (low temperature) were used for Mg/Ca to temperature calibration (Moberly 1970; Corrège 2006; Williams *et al.* 2014). The Mg/Ca values were plotted against the average temperature values for August and January selected from multiple locations and depths around the sampling area from WOA01, Quarter Degree Resolution (the World Ocean Atlas 2001, Fig S1). Only Recent specimens were used for the calibration since the palaeo-temperature is not known. The temperature used represents the depth of sampling. For *L. minervae* the depth of the collection site and the depth of the temperature taken from the temperature transect differ by 6 meters while for *L. stictaeforme* temperature taken from the temperature transect differ by 2 meters.

In the species-specific calibration, the spatial variability of the Mg was used to establish seasonal cyclicity with low Mg/Ca values tied to winter (February is the coolest month) and highest Mg/Ca to summer (August is the warmest month). Additionally, distance between two Mg/Ca minimum was used to estimate annual growth rate.

In order to compare the Mg content between the different samples of *L. minervae* and *L. stictaeforme*, a Kruskal-Wallis analysis was performed (Corder *et al.* 2014). All Pairwise Multiple Comparison Procedures (Dunn's Method) were used to identify which samples were significantly different. A Mann-Whitney Test (Mann and Whitney 1947) was used to test for differences between the recent and the subfossil sample of *M. philippii* and a Kruskal-Wallis analysis for the comparison between samples of the same age (Dinno 2015).

$\delta^{11}B$ determination-pH reconstruction

Boron isotopes of marine carbonates are an established proxy for sea water pH reconstruction (e.g. Hemming and Hanson 1992; Hönisch and Hemming 2005; Pelejero *et al.* 2005; Foster 2008). Boron has two stable isotopes, ^{11}B and ^{10}B, that make up ~80% and 20%, respectively. Boron exists in sea water as two molecular species: boric acid ($B(OH)_3$, trigonal) and borate ion ($B(OH)^{4-}$, tetrahedral), connected by the boric acid equilibrium (Zeebe and Wolf-Gladow 2001) (eq. 2):

$$B(OH)_3 + 2H_2O \rightleftharpoons B(OH)_4^- + H_3O^+ \quad (1) \qquad (2)$$

The distribution of these species is pH controlled. Between both species, a strong equilibrium fractionation of 27.2‰ exists for boron isotopes (Klochko *et al.* 2006), boric acid being isotopically heavier and borate ion lighter. Thus, as pH changes the relative abundances of both species and the isotopes are distributed in a way to maintain both, the total boron isotope

composition (when integrated) and the isotopic offset mentioned above. This results in not only the abundances but also the isotopic compositions of both species of boron in seawater being pH dependent. Since being established as a proxy (Hemming and Hanson 1992) the basic assumption has been that only the borate ion is incorporated into the carbonates formed in the marine environment. Offsets between the boron isotopic composition of those carbonates and borate ion in sea water have been interpreted as "vital effects" (Hönisch and Hemming 2005).

Under the previously mentioned assumptions (only borate ion $B(OH)^{4-}$ being incorporated into marine carbonates) ocean pH can be calculated from marine carbonates' $\delta^{11}B$ according to eq. 3:

$$pH = pK_B^* - log\left(-\frac{\delta^{11}B_{SW} - \delta^{11}B_{CaCO_3}}{\delta^{11}B_{SW} - \alpha_B \cdot \delta^{11}B_{CaCO_3} - 1000 \cdot (\alpha_B - 1)}\right) \quad (3)$$

where pK_B is the equilibrium constant between $B(OH)_3$ and $B(OH)^{4-}$(pK_B =8.906-0.0123*T, Dickson 1990) $\delta^{11}B_{CaCO3}$ is the boron isotopic composition of the carbonates (considered to be the $\delta^{11}B$ of $B(OH)^{4-}$ with a variable contribution from species-specific "vital effects" (Hönisch *et al.* 2007), $\delta^{11}B_{sw}$ the boron isotopic composition of seawater (39.61‰; Foster *et al.* 2010) and α_B the fractionation factor between $(OH)_3$ and $B(OH)^{4-}$ (1.0272; Klochko *et al.* 2006).

Boron isotopes were measured by Laser Ablation-Multicollector-Inductively Coupled Plasma-Mass Spectrometry (LA-MC-ICP-MS) following the procedure described by Fietzke *et al.* (2010). Prior to the measurements and the preparation of the sample, the organic matter was removed by immersion in a solution of diluted hydrogen peroxide (<1%) for 24 hours (Mertz-Kraus *et al.* 2009) and cleaned ultrasonically. All areas to be analysed were pre-ablated to remove contaminations from the sample surface. The NIST610 was measured using 25 µm spot, while samples were measured at 50 µm (repetition rate of 10 Hz, 40 s of ablation). Data collection was performed in multi-ion-counting mode at mean ^{11}B intensities of about 100.000 cps (internal $\delta^{11}B$ error for individual runs ~1.2 ‰ 2SD). Each sample result consists of data of 18 runs (to improve the counts and reduce the analytical error), accompanied by 27 repeated determinations of NIST610 as standard, which yielded a $\delta^{11}B$ of 0.55±0.33 ‰ 2SD.

In order to compare the $\delta^{11}B_{CaCO3}$ between the different samples of *L.minervae* and *L.sticateforme* and *M. philippii* a Kruskal Wallis analysis and t-test were performed (Corder *et al.* 2014). All pairwise multiple comparison procedures (Dunn's Method) were used to identify which samples were significantly different when needed.

RESULTS

Growth patterns and rates (thickness)

Modern *L. minervae* (T) displays the highest annual growth rates (Table 1), while *L. stictaeforme* (ME) has the lowest (Table 1), even though the species were collected at similar depths. This difference in growth rates reflects the different growth patterns. The lack of clear Mg/Ca cyclicity in *M. philippii* (ME&T) hindered the determination of growth rates for the modern sample. In the fossil specimens from 8.6 cal ka BP, *L. stictaeforme* (ME) and *M. philippii* (ME&T) have similar growth rates (Table 1) and patterns while the fossil specimens from 11.37 cal ka BP *L. minervae* (T) and *L. stictaeforme* (ME) show different growth rates with the highest annual growth rates in *L. miervae* (Table 1) reflecting the different growth patterns.

Mg content and temperature reconstructions

Within the live-collected specimens, the average Mg content in *L. minervae* is significantly (Kruskal-Wallis, $p<0.001$, df=2, H=110.78) higher than in *L. stictaeforme* and *M. philippii* (Fig. 3b). The 8.6 cal ka BP specimens with similar growth patterns of *M. philippii* and *L. stictaeforme*, (12.88±2.4;12.53±1.28 molMg% respectively) collected at the same palaeodepth, have similar Mg concentrations (Mann-Whitney, p=0.67). Similarly, 11.37 cal ka BP specimens of *L. minervae* 11.86±0.97;11.79±0.51 molMg% respectively and *L. stictaeforme* collected at the same palaeodepth but with different growth patterns have the same Mg concentrations (Kruskal-Wallis, p =0.24, df=2, H=0.834). Therefore, the average concentration of Mg in the coralline algae skeleton does not seems to be influenced by the growth patterns.

Internal variability of Mg/Ca which we consider seasonal variation in accordance with the literature of other species, is, however, influenced by the growth pattern. The recent material shows differences in Mg/Ca variability in all three genera. There is a high internal Mg/Ca variability in *L. minervae (*0.07 ± 0.003 $Mg/Ca_{mol/mol}$), a low one in *L. stictaeforme* (0.01 ± 0.006 $Mg/Ca_{mol/mol}$), and no clear patterns in *M. philippii* (Fig. 4). *L. minervae* is the only species that shows distinct cyclical variation in Mg/Ca in all the samples analysed (10.6 cal ka BP: 0.039 ± 0.02 $Mg/Ca_{mol/mol}$; 11.37 cal ka BP: 0.024 ± 0.015 $Mg/Ca_{mol/mol}$). While Mg/Ca was stable in the recent specimen of *M. philippii*, the sample from 8.6 cal ka BP shows Mg/Ca variability (0.052 ± 0.003 $Mg/Ca_{mol/mol}$ overall and 0.031 ± 0.006 $Mg/Ca_{mol/mol}$ for the younger part-160 µm of the specimen). *L. stictaeforme* shows Mg/Ca variability in both the

fossil samples, with the 8.6 cal ka BP being higher (0.036 ± 0.07 Mg/Ca $_{mol/mol}$) than the 11.37 cal ka BP sample (0.015±0.006 Mg/Ca $_{mol/mol}$).

We used the species-specific temperature dependency of the Mg incorporation determined in the modern specimen of *L. stictaeforme* and *L. minervae* (Table 2) to try to reconstruct the palaeo-temperature from the fossil Mg values. The lack of seasonal variation in *M. philippii* hinders us from applying the same approach. Relationships are presented both as mol % and ratios to Ca (Table 1). All element–temperature relationships are linear and positive. Mg-SST (Mg/Ca) relationships in both species have R2 values >0.88 while the Mg-SST (mol%) relationship had a lower R2 (>0.77).

pH reconstructions

The $\delta^{11}B$ measurements (Fig. 5) of both recent specimens of *L. minervae* (23.8 ± 0.7 $\delta^{11}B$), *L. stictaeforme* (25.3 ± 0.5 $\delta^{11}B$) and *M. philippii*, (25.9 ± 0.4 $\delta^{11}B$) and fossil specimens (8.6 cal ka BP: *L. stictaeforme* 21.9 ± 0.6 $\delta^{11}B$; *M. philippii* 21.9 ± 0.9 $\delta^{11}B$, 10.6 cal ka BP: *L.minervae* 19.8 ± 0.4 $\delta^{11}B$, 11.37 cal ka BP: *L.minervae* 24.4 ± 0.6 $\delta^{11}B$; *L. stictaeforme* 23.7 ± 0.7 $\delta^{11}B$) show the same trend (Fig 5b) with lower pH at 8.6 cal ka BP and 10.6 cal ka BP compared to modern samples and a subsequent increase to modern pH at 11.37 cal Ka BP.

There was no significant different between the modern specimens apart from *M. philippii* and *L. minervae* (one way ANOVA; p = 0.035). *L. minervae* showed a significant decrease from modern to 10.6 cal Ka BP and an increase to modern value at 11.37 cal Ka BP (One Way ANOVA. recent vs 10.6 cal Ka BP p<0.001; 10.6 cal Ka BP vs 11.37 cal Ka BP p<0.001; recent vs 11.37 cal Ka BP p= 0.507). For the *L. stictaeforme* the same trend is visible with a significant decrease from modern to 8.6 cal Ka BP and an increase to the modern value at 11.37 cal Ka BP (One Way ANOVA. Recent vs 8.6 cal Ka BP, p<0.001; 8.6 cal Ka BP vs 11.37 cal Ka BP p=0.1; recent vs 11.37 cal Ka BP p= 0.08). The two specimens of *M. philippii* again showed the same trend, with a significant decrease at 8.6 cal Ka BP (Mann Whitney p= 0.012).

DISCUSSION

Our assessment of the use of coralline algae for palaeoenvironmental reconstruction in subtropical settings is promising. The similarity of the Mg/Ca ratios in our material though could also be driven by post-depositional alterations. As such, assessing preservational bias is fundamental to the interpretation of the data. Post-mortem skeletal alteration of coralline thalli

may lead to erosion and corrosion, thereby increasing porosity, and typical a loss of Mg, turning the high-Mg calcite into low-Mg calcite during fossilization. None of these modifications is obvious in our specimens. Overall, carbonate mineralogy among the coralline algae from the same geographical region is broadly similar due to the influence of temperature in the incorporation of Mg^{2+} and therefore trends with age would be expected. Our results show no correlation between age and Mg content. The three investigated genera have lower average Mg concentrations (~11-15 mol% Mg) compared to the same genera in the tropics (~16-23 mol% Mg), matching the expected impact of the temperature differences of the area they lived in (Clark and Wheeler 1922; Smith *et al.* 2012).

In this study we used species of two different orders of the subclass Corallinophycideae, (Hapalidiales: *Lithothamnion minervae* and *Mesophyllum philippii* and Corallinales: *Lithophyllum stictaeforme*) which showed different thickening growth rates in response to their growth pattern (T, ME and ME&T) in both recent and fossil specimens. Changes in species growth patterns have the potential to affect growth rates, but our data suggest that there is no overall link between annual average growth rate and Mg incorporation in these species.

Fossil samples show similar Mg content at the same palaeodepth suggesting no significant temperature difference between species. Through time though we show the potential to reconstruct the shallow water coastal environments. Our samples from 8.6 cal ka BP suggest temperatures similar to the modern, in agreement with the reconstruction of the Holocene climate in the Tyrrhenian Sea (Alley *et al.* 1997; Kallel *et al.* 1997; Buccheri *et al.* 2002; Sbaffi *et al.* 2004). So far the TC6 cooling event (Cacho *et al.* 2001) has only been documented in high resolution marine records (Kallel *et al.* 1997; Buccheri *et al.* 2002; Sbaffi *et al.* 2004). Our data though for the first time provides evidence for the cooling event in the shelf ecosystems with the lowest temperatures at 10.6 cal ka BP and warmer temperatures at 11.37 cal ka BP. These results show the potential for temperature reconstructions based on a sound taxonomic framework and understanding of the habitat.

In contrast to the open ocean core-based reconstructions, we can additionally assess changes in seasonality by interpreting the pronounced cyclic variability in algal Mg/Ca as a seasonal cycle. We suggest that the growth pattern influences if the seasonal temperature variability is recorded or not. Only *L. minervae* which grows by thickening recorded oscillations in Mg concentrations in all samples (Fig. 4). *L. stictaeforme* and *L. minervae* were sampled at similar water depth, showing that habitat depth alone cannot be the cause for the

lack of variability in one versus the other. *L. stictaeforme* grows mainly by marginal elongation and as such the lower growth rates in response to the predominantly marginal growth lead to a loss in resolution in the element variability along an ideal transect of the thickening, as also observed in *Clathromorphum* spp. (Halfar *et al.* 2011; Hetzinger *et al.* 2011), and *Lithophyllum yemenense* (Caragnano *et al.* 2014, 2017). The growth pattern therefore creates the impression of an environmental stability which does not reflect the reality. *M. philippii*, the deepest among the living specimens, grows with a combination of marginal elongation and some moderate thickening. Due to the deep habitat of 97 m, an absence of Mg/Ca cyclicity in the deep *M. philippii* is expected, as temperature only varies by 0.27± 0.08 degrees. Understanding the growth pattern will allow differentiating between the influences of growth *versus* habitat depth, and hence environmental stability, in recording the seasonal variation in fossil material. Our results suggest that *L. minervae* is a good recorder of temperature and its seasonality, an interpretation further supported by the observation that another species of the same genus, *L. glaciale,* is extensively used for palaeotemperature reconstruction (Kamenos *et al.* 2008).

Additional to the overall growth habit of a species, changes in growth patterns during the life history of a species can influence its reliability as a seasonality recorder. Based on this study, though, there is information which can be drawn from fossil material if the growth patterns are well understood. The *M. philippii* specimen from 8.6 cal ka BP shows a very irregular growth variation in the oldest part of the record, but established a more regular pattern in the younger part. We suggest that the marginal growth was dominant during the early stage of thallus development by fast substrate coating, leading to a loss in resolution comparable to *L. stictaeforme.* Interpreting the Mg/Ca variability exclusively in the youngest part of the thallus of *M. philippii* (Fig. 4), the mean observed amplitude is similar to *L. stictaeforme* of the same age and depth.

The samples of *L. stictaeforme* and *L. minervae* from the TC6 cold event (Fig. 3; Cacho *et al.* 2001) record slightly different environmental information. *L. minervae* has lower Mg content and lower seasonal variation in these samples compared to the recent, which might reflect the increased palaeodepth of about 60 m compared to 44 m (Table 1) and thereby lower temperatures and seasonal variation. *L. stictaeforme,* in contrast, recorded the same seasonal temperature variation as today, despite the difference in water depth. We are not able to determine if this is a physiological response such as different sensitivity to light attenuation or other environmental changes driven by the change in water depth associated with the TC6

cooling event. It does raise concerns though about how faithful seasonality can be derived and needs to be assessed in a larger dataset of well dated specimens covering the event.

The power in combining the temperature record with a boron isotope record is the potential to link changes in two main drivers of growth of calcifiers. While boron is faithfully recorded by a number of species, its uptake is strongly influenced by species physiology such as calcification mechanisms, internal up-regulations and photosynthesis (Hönisch *et al.* 2003; Trotter *et al.* 2011; McCulloch *et al.* 2012, Cusak *et al.* 2015). Therefore the skeletal $\delta^{11}B$ within the specimen is often significantly higher than measured ambient pH and the mechanisms need to be established for each new carrier species. Recently it has been shown that this physiological offset may be species specific in coralline algae (Cornwall *et al.* 2017). So far no $\delta^{11}B$–pH calibration exists for these coralline algae.

In this study, absolute boron-derived pH values are ~0.3 to 0.4 pH units above the ambient seawater pH range in the Tyrrhenian Sea for the corresponding depth (Hassoun *et al.* 2015) suggesting a biological control on the pH in the calcifying fluid similar to *Clathromorphum nereostratum* (Fietzke *et al.* 2015). A comparable offset between $\delta^{11}B$-derived and ambient seawater pH has also been observed in other marine calcifying organisms (McCulloch *et al.* 2012; Holcomb *et al.* 2014; Wall *et al.* 2015). McCulloch *et al.* (2012) suggest that species either exhibit a low-sensitivity response consistent with strong pH up-regulation or a higher sensitivity due to an inability to up-regulate pH, resembling abiotic calcite (McCulloch *et al.* 2012).

Our inferred pH reconstruction for the Mediterranean Sea at 11.37 cal ka BP showed values similar to present. In contrast, at 8.6 and 10.6 cal ka BP all analysed species recorded lower pH compared to recent specimens, contrarily to what would be expected based on the lower atmospheric CO_2 at that time (Monnin *et al.* 2004). There are two potential explanations for this counterintuitive result, physiological changes related to compounding factors such as light availably for the algae photosynthesis, which could impact the upregulation as described in other calcifiers (Köhler-Rink and Kühl 2005; Rink *et al.* 1998), or local changes in pH. Coastal waters are often not in equilibrium with the atmosphere (Artioli *et al.* 2014). Therefore, the low pH values may reflect regional changes in pH due to the rapid drowning of the shelf and the sill between Ponza and Palmarola Islands, limiting the exchange with the open ocean (Corselli *et al.* 1994) and changing pH and alkalinity. The relatively rapid inundation of a nearby sizable area of land (Corselli *et al.* 1994), probably vegetated during the LGM and

therefore organic rich, would have changed the local chemo-physical conditions, and hence potentially lowered the seawater pH in response to the oxidation and decay of the organic matter.

CONCLUSIONS

The aim of this study was to assess the most suitable genus for palaeoclimate reconstructions of shelf settings in the Mediterranean. The incorporation of Mg in the carbonate thallus of the three investigated algae is primarily driven by ambient temperature. However, changes in growth patterns during the life history of the species may influence its reliability as a seasonality recorder. Therefore growth patterns of the species need to be well understood prior to analysis. The Mg/Ca variation within each specimen displays seasonal variability of temperature, the detectability of which is favoured by the amount of $CaCO_3$ deposited during growth (T). The recent and fossil specimens of coralline algae showed a consistent pattern of boron incorporation, thus opening new perspectives as proxy of pH variability in shelf areas. Overall, *L. minervae* seems to be the best biogenic archive of palaeoenvironmental proxies among the taxa investigated here, allowing for the combination of reconstructions of temperature, its seasonality and pH in the same species. As the genus has a temperature range from 6 to 30°C a wide geographical area can be reconstructed which, combined with its long geological range since the Cretaceous (Aguirre *et al.* 2010), make it an ideal genus for climate reconstructions in coastal settings.

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Table captions

Table 1. Location, growth patterns and rates, year and depth of both recent and fossil specimens used in this study. T growth pattern: mainly thickening, ME growth pattern: mainly marginal elongation and ME&T growth patterns: both marginal elongation and thickening. Growth rates measure are in µm ± SD (n=4).

Table 2. Proxy-temperature relationships of *Lithothamniono minervae* and *Lithophyllum stictaeforme*.

Figure captions

Fig. 1. Picture of the samples taken into consideration during this study: A, D, G: *Lithothamnion minervae* (T); sample numbers: A = DB664, D = DB668, G = DB671. B, E, H: *Lithophyllum sticateforme* (ME); sample numbers: B = DB665, E = DB669, H = DB670. C, F: *Mesophyllum philippii* (ME&T); sample numbers: C = DB666, F = DB667. T = growth pattern, mainly thickening, ME = growth pattern, mainly marginal elongation, ME&T = growth pattern, both marginal elongation and thickening. All scales represent 0.5 cm.

Fig. 2. Position of grab, dredge and core samples around Palmarola Island during the Cruises MINE89 and MINAU89. Regional section highlighted (red square) on the map of Italy in the inset.

Fig. 3. Sea Surface Temperature (SST) reconstruction for the Western Mediterranean during the Holocene (A) from Cacho *et al.* (2001). Mg content (mol% Mg) of *Lithothamnion minervae*, *Lithophyllum stictaeforme* and *Mesophyllum philippii* (B) and reconstructed temperature (°C) for *L. minervae* in recent, 8.6 cal ka BP, 10.6 cal ka BP and 11.37 cal ka BP samples (C). TC arrows indicate the position of cold Holocene events. The blue band shows the Younger Dryas cooling event (YD). Age Uncertainty ± 50 years. (Error bars= Standard error; n = 105). Dashed line reprepsent the Cold spells TC5 and TC6.

Fig. 4. Time series of $Mg/Ca_{mol/mol}$ ratio from recent and fossil speciemens of the three coralline species: A = *Lithophyllum stictaeforme*, B = *Mesophyllum Philipii*, C = *Lithothamnion minervae*. Error bars in the top right corner of each graph indicate the

uncertainty based on the counting statistics. Measurements were taken along the growth direction.

Fig. 5. A, the relationship between CO_2 concentration in the atmsphere and pH in the ocean. Increase in CO_2 (CO_2 concentration in the atmosphere during the Holocene was taken from Monnin *et al.* (2004)). B, $\delta^{11}B$ and the reconstructed pH *of Lithothamnion minervae*, *Lithophyllum stictaeforme* and *Mesophyllum philippii* in recent, 8.6 cal ka BP, 10.6 cal ka BP and 11.37 cal ka BP samples. (Error bars = standard error - pH; n=17). The blue band shows the Younger Dryas cooling event (YD).

Fig. S1. Modern water parameters of the sampling site. Location, depth and temperature for January and August extrapolated from the World Ocean Atlas 2001. The data were used for the temperature calibration of the recent species.